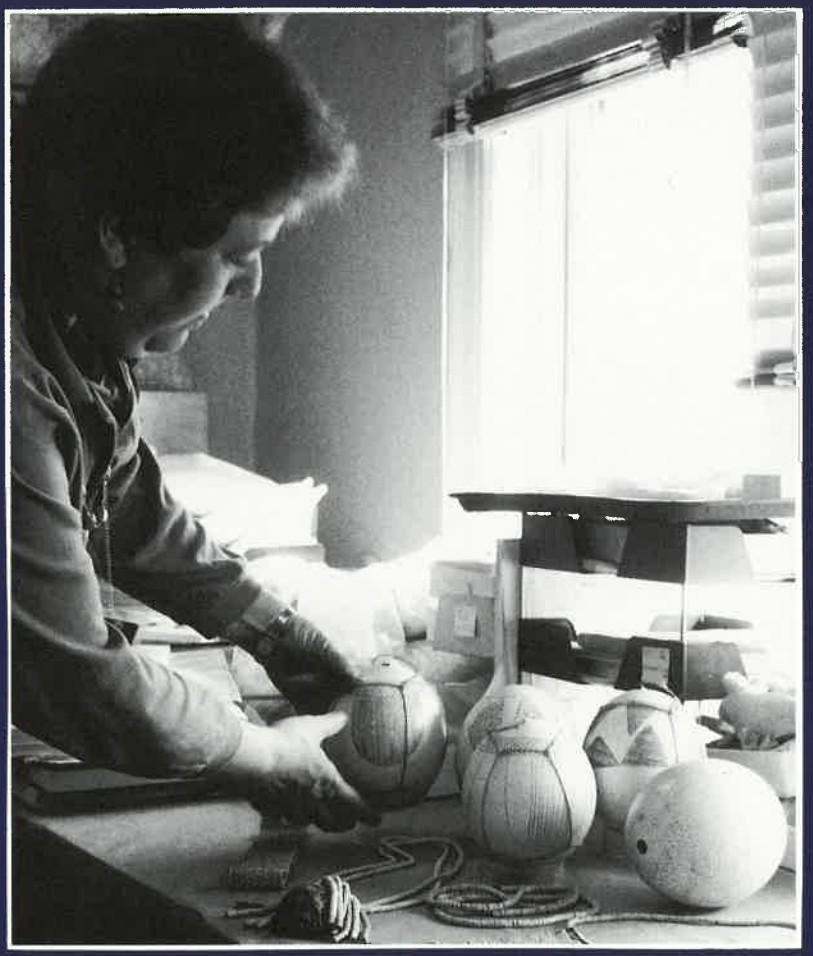

The Chemistry of Ancient Life

amino acids, stable isotopes, and the anthropology connection

The Chemistry of Ancient Life

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by Patricia Parratt Craig

an essay about basic research from
the Carnegie Institution
of Washington

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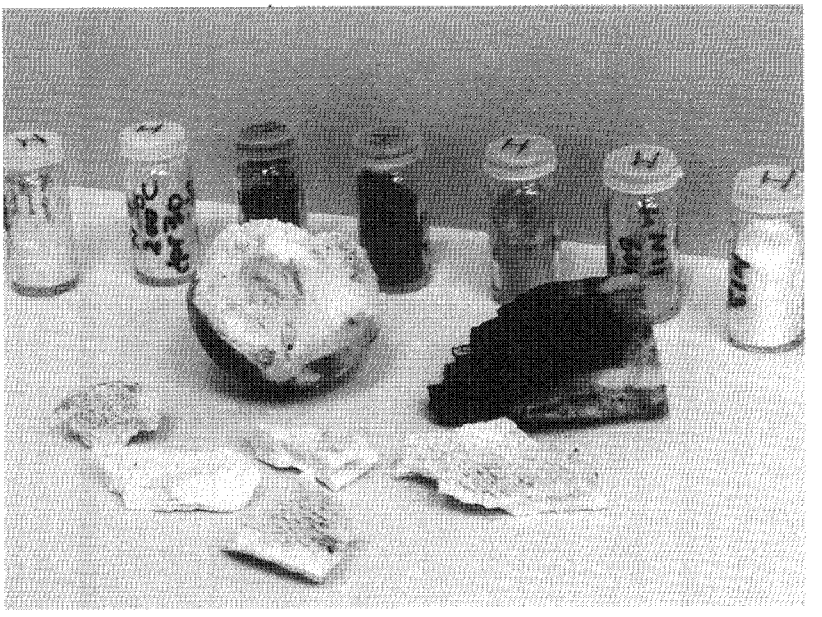
Preface

Founded in 1902 by steel magnate and philanthropist Andrew Carnegie, the Carnegie Institution was envisioned a place where the "exceptional" individual might find opportunity for a lifetime of discovery. Today, such individuals work within a five-department structure that encompasses basic research in astronomy, biology, and the earth sciences. Scientists at one of those departments, the Geophysical Laboratory, study questions about the Earth's chemistry and evolution. The following essay explores how a handful of the scientists there—the biogeochemists—are also applying their tools to questions of human prehistory.



Contents

Introduction 5

Amino Acids as Dating Tools: Using Ostrich Eggshells to Date Hominid Sites 7

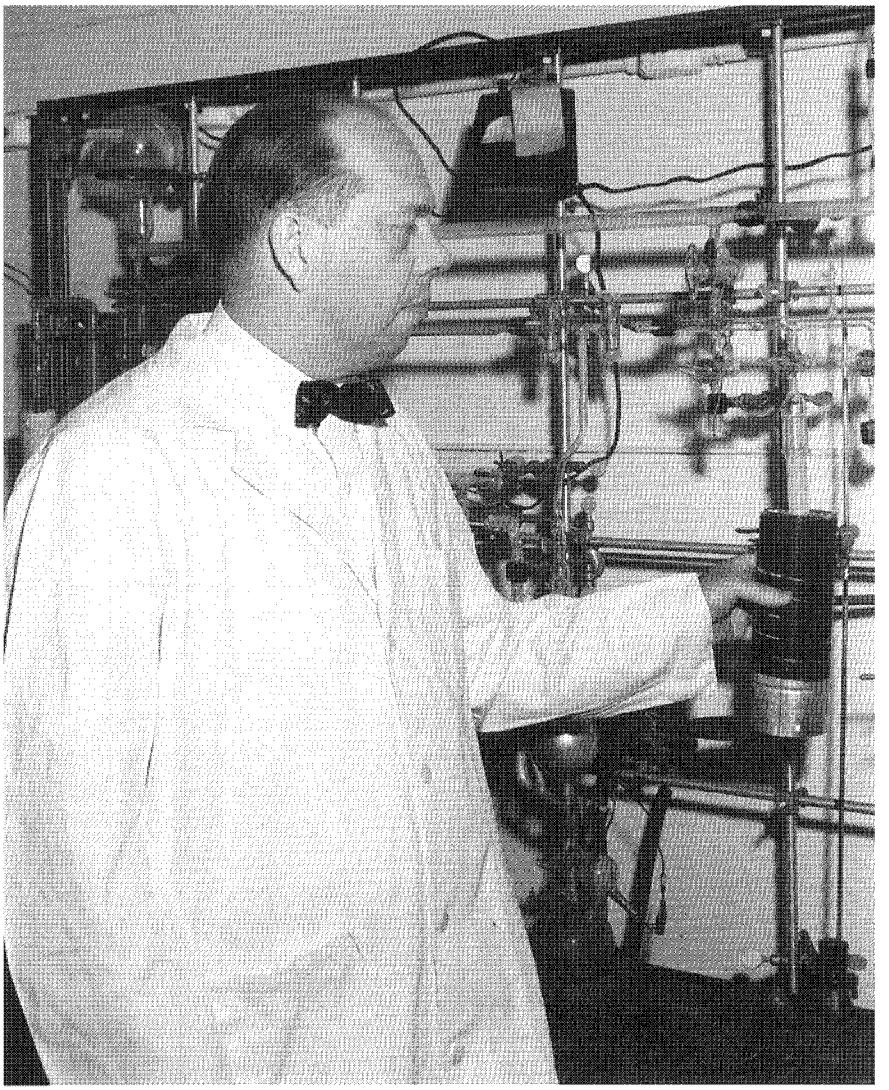
- Are Neandertals Our Ancestors? 7
- A "Serendipitous" Discovery 9
- The Eggshell Project 14

Isotopes as Dietary Tracers: Studying Nursing Patterns Among Prehistoric Peoples 21

- You Are What You Eat 22
- Finding a Nursing Signal 26

Studying How Bones Decay 30

Conclusion 39



Philip Abelson, c. 1955, at the Geophysical Laboratory

"...although the outward forms of animals have changed, the principal inner chemical mechanisms of today are similar to those employed hundreds of millions of years ago."

Philip Abelson
1963

Some forty years ago, an inquisitive scientist crushed a handful of 25-million-year-old clam shells, dissolved them in a weak acid solution, and attempted to extract amino acids—the building blocks of protein. The scientist wondered if the ancient amino acids could possibly have survived during their long burial in the Earth. There was no good reason to suppose they had. At the time, fossils were known to preserve only the shapes of ancient creatures; no one thought they preserved any evidence of their chemistry. To study such matters, it was believed necessary to look at living organisms, particularly those whose appearance differed little from their fossil ancestors.

But Philip Abelson was lucky. He was able to extract seven amino acids from the fossilized clam shells, thereby proving that some amino acids can indeed survive burial for geological periods of time.

Abelson and his colleagues later performed laboratory experiments on proteins extracted from the shells of living clams. They hoped to learn what happened to the proteins in the presence of water and high temperatures—conditions simulating burial in the Earth over long periods of time. Incubating modern shells for ten days at 170°C or 70 days at 143°C, they found, had approximately the same effect as 25 million years at around 15°C.

The investigators then detailed the sequence by which the proteins degraded. First, water—ubiquitous in nature and necessary for protein decay—penetrates the animal's shell, breaking down the shell's proteins into peptide chains and individual amino acids. This step is called hydrolysis. Some of the amino acids then leach out of the shell. Those remaining in the shell gradually lose their integrity and change their chemical organization slowly with time; the higher the temperatures, the faster the changes. Abelson determined that a substantial amount of one amino acid—alanine—should remain in a shell if kept at room temperature for about a billion years. His work thereby indicated not only that organic geochemical processes could

Top to bottom: Ed Hare, Marilyn Fogel, and Paul Koch—the biogeochemists at the Geophysical Laboratory featured in this essay.

be investigated in the laboratory, but that amino acids might be useful markers in studying biological organisms through geological time; older fossils, for example, should be rich in the most stable amino acids but contain relatively little (or none) of the least stable.

At the time of his discovery, in the early 1950s, Philip Abelson was director of the Carnegie Institution's Geophysical Laboratory—a research lab in Washington, D.C., dedicated to the study of the Earth. Nearly twenty years later he was to become president of the Carnegie Institution and, at the same time, editor of the journal *Science*. He was, and still is, one of the most well-known, outspoken, and accomplished scientists of his time.

After his discovery, Abelson spent several years at the Laboratory continuing experiments in the field of what is now informally called biogeochemistry. But, for the most part, he left the later development of the field to others. Biogeochemistry soon came to include the analysis of the stable isotopes of such elements as carbon, nitrogen, and oxygen—isotopes discovered by scientists at the Laboratory and elsewhere to contain a record, like amino acids, of the ancient past. Today, the Geophysical Laboratory remains at the forefront of biogeochemical research. Of some fifteen staff members there, three—Ed Hare, Thomas Hoering, and Marilyn Fogel—call themselves biogeochemists. They lead a program of, at any one time, from eight to ten postdoctoral fellows, predoctoral fellows, and visiting investigators who spend anywhere from a few months to several years at the Lab before beginning their professional careers elsewhere or returning to their original institutions.

Today, the field is extraordinarily diverse. Many biogeochemists work in the petroleum industry, for the tools of the biogeochemist can yield information about the transformation of organic debris to deposits of oil and gas. Others, working in commercial laboratories, use the tools to monitor the purity of certain foods. Still others, working at universities and places like the Geophysical Laboratory, are involved in more-esoteric studies, learning, for example, the details by which the process of organic decay proceeds.

In recent years, scientists have begun applying biogeochemical techniques to such issues as global change, animal preservation, and archaeology. The following essays concentrate on one of these aspects of biogeochemistry as practiced at the Geophysical Laboratory—the use of amino acid and stable isotope techniques to elucidate certain questions of anthropology and paleontology. For, as we will learn, the study of organic matter in the fossil record is yielding new insights into long-puzzling questions, such as the emergence of modern humans, the day-to-day life of prehistoric peoples, and the extinction of mammoths and other large mammals at the end of the last great ice age 10,000 years ago.

Amino Acids as Dating Tools:

Using Ostrich Eggshells to Date Hominid Sites

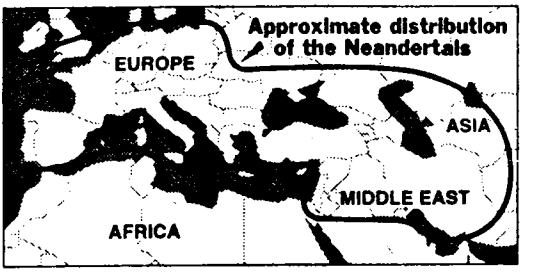
Sitting amid the chemical separation instruments, amino acid analyzers, and gas chromatographic machines in Ed Hare's lab is a newsletter about—of all things—ostriches. To the first-time visitor, this might seem strange. But to Hare and his colleagues, ostriches are serious business. They are the source of a material recently found to offer a means for dating archaeological sites. Hare and his collaborators, including anthropologist Alison Brooks, a visiting investigator from the George Washington University and the Smithsonian Institution, are learning how to "read" amino acids in fossilized ostrich eggshells. They are looking for new clues about the habitation sites of ancient humans.

Are Neandertals Our Ancestors?

The story begins from 100,000 to 200,000 years ago, when anatomically modern humans—our direct ancestors—first appeared on the Earth. The event is shrouded in mystery, for no one can say for sure exactly when or where this happened. Did modern humans first appear in Africa, as many anthropologists now believe, and then migrate throughout the rest of the world? And, if so, when and with what consequences did they encounter the more primitive peoples—the Neandertals—whom they eventually replaced?

Neandertals displayed robust skeletal features such as bulging brows, thick bones, and projecting jaws. Though their brains were as large or larger than the brains of anatomically modern humans, the cultural artifacts they left behind are much simpler. The earliest Neandertal fossils, found in Europe, date to about 250,000 years ago. By 80,000 years ago, Neandertals lived also in central Asia and the Middle East. Some 45,000 years later, about 35,000 years ago, they were all but gone.

Until recently, most anthropologists assumed that Neandertals and their close relatives in Asia and Africa were an intermediate, transitional form linking *Homo erectus* (an earlier, even more primitive ancestor) and modern humans. In this view, modern humans evolved from the Neandertals.



But new evidence from an array of high-tech, often controversial dating techniques suggests that modern humans did not evolve from Neander-

tals. Instead, the two may have been branches of the same family tree that co-existed for thousands of years. The evidence is particularly puzzling in the Middle East, where some modern human fossils appear actually to *predate* many Neandertal fossils. Also, some Neandertal-like bones from the Middle East appear to be less robust than those unearthed in Europe. Does this mean that modern humans and Neandertals interbred in the Middle East, producing a hybrid form?

At this point, no one can say. No one knows the sequence of events leading to the disappearance of the Neandertals. No one knows, for example, how long they may have co-existed with modern humans, or if they exchanged tools and ideas, perhaps even genes. According to anthropologists, answers to these questions require, above all, a better chronological framework in which to study habitat and migration patterns. And this, in turn, requires better ways to date fossils.

Radiocarbon dating is anthropology's most common dating method. It measures the decay of the radioactive isotope ^{14}C to the element nitrogen. Radiocarbon dating, however, is reliable only on fossils that are younger than about 35,000 years. (The half-life of ^{14}C is 5730 years; thus, half of the ^{14}C is decayed after 5730 years, half of the remaining ^{14}C decays after another 5730 years, and so on. After 35,000, or about six half-lives, the levels of ^{14}C are so low that contamination becomes a serious problem in analysis.) Another proven technique—potassium-argon dating (based on the decay of the radioactive isotope potassium ^{40}K to the inert gas argon ^{40}Ar)—has generally been used only on samples that are older than 300,000 years. For fossils that fall between 35,000 and 300,000 years of age, there is no proven dating method. This has been unfortunate: it is precisely within this time that anatomically modern humans emerged.

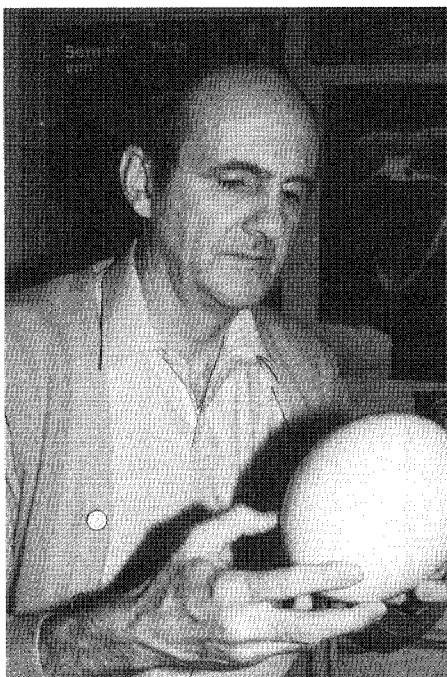
Several new, high-tech dating techniques have recently been developed that promise to fill the 35,000–300,000-year gap. But these techniques, along with recent improvements in radiocarbon and potassium-argon dating that have extended their ranges, are still in experimental stages. Two of the most widely used of the new, high-tech methods—thermoluminescence (TL) and electron spin resonance (ESR)*—are derived from solid-state physics. Applied to samples of

*TL and ESR measure time by testing for tiny, radiation-induced changes in mineral crystals (rocks, tooth enamel, etc.). The crystals trap energy from radiation in the soil. As time goes on, more and more energy is absorbed until all the "traps" that can store the energy are full. To date the crystal by TL, one then heats the sample and measures the amount of trapped energy that "escapes" as light. To date by ESR, one measures the trapped energy magnetically. (In a third technique closely related to TL, called optically stimulated luminescence, the trapped energy is released not by heat but by laser light.)

burnt flint found at archeological sites, TL and ESR suggest how much time has passed since the flint was last heated. Both have provided evidence that anatomically modern humans lived on the Earth longer than anyone would have guessed even a decade ago. But the techniques have drawbacks. A piece of flint may, for example, have been part of a fireplace structure at an early hominid site. But if that flint was heated subsequently, its zero point would have been reset and it would become useless for dating the original site.

Another recent and even more controversial technique, borrowed from the biological sciences, measures the variation in mitochondrial DNA among modern species or populations, thereby indicating how long ago the species or populations diverged. (Mitochondria are small intracellular organelles that contain a special kind of DNA inherited only from the mother.) In 1987, a group of researchers at the University of California, Berkeley, produced a family tree of human origins based on mtDNA analysis. They claimed that the ancestry of all modern humans could be traced to a single individual, a single "Eve," who lived in Africa 100,000–300,000 years ago. Then, in early 1992, other researchers showed that the statistical techniques used in the original mtDNA analysis were flawed. By that time, however, the mtDNA work had already sparked vigorous debate among anthropologists, many of whom were already drawing the same conclusion—an African origin for modern humans—from paleontological evidence.

Amid such controversial techniques and claims, Hare, Brooks, and their collaborator Gifford Miller at the University of Colorado, Boulder, discovered that fossilized ostrich eggshells carry their own chemical clock, seen in the decay of amino acids over time. This technique, called amino acid racemization (or AAR), is not, technically, new, nor is it unknown to anthropologists. During the 1970s, anthropologists used AAR to date fossil human bones. The results were exciting. But for reasons to be made clear below, anthropologists soon became distrustful of the technique, and it returned wholly into the geologist's domain. Now, applied to fossilized ostrich eggshells, AAR is making an anthropological comeback.

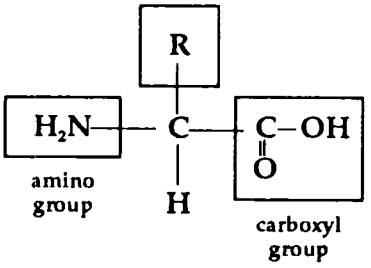


In Ed Hare's many years at the Geophysical Laboratory, he has had several offers to go elsewhere to teach. But he's turned them all down. "There's no substitute for being able to go into the lab and do what you want," he says. Above, he holds an ostrich eggshell.

A "Serendipitous" Discovery

Ed Hare has been at Carnegie's Geophysical Laboratory in Washington, D.C., since 1963. He was

WHAT IS AN AMINO ACID?



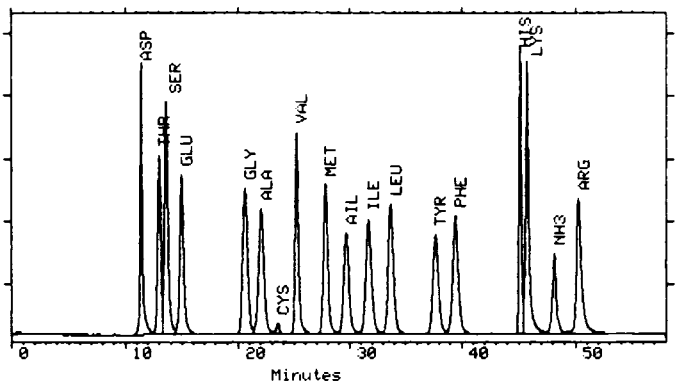
Amino acids are the subunits of polypeptide and protein chains. Of the twenty amino acids that exist in proteins, some can be synthesized by an animal, while others must be consumed. The structure of all amino acids is similar. Each consists of a central carbon surrounded by four units: a carboxyl (acid) group, an amino group, a single hydrogen atom, and an "R" chain. The R chains are made of various combinations of carbon, hydrogen, oxygen, and nitrogen, and are what distinguish individual amino acids. Amino acids are linked by what is known as a peptide bond, joining the carboxyl group of one amino acid to the amino group of another. An average peptide chain consists of several amino acid units. Protein molecules are larger.

Separating amino acids by chromatography

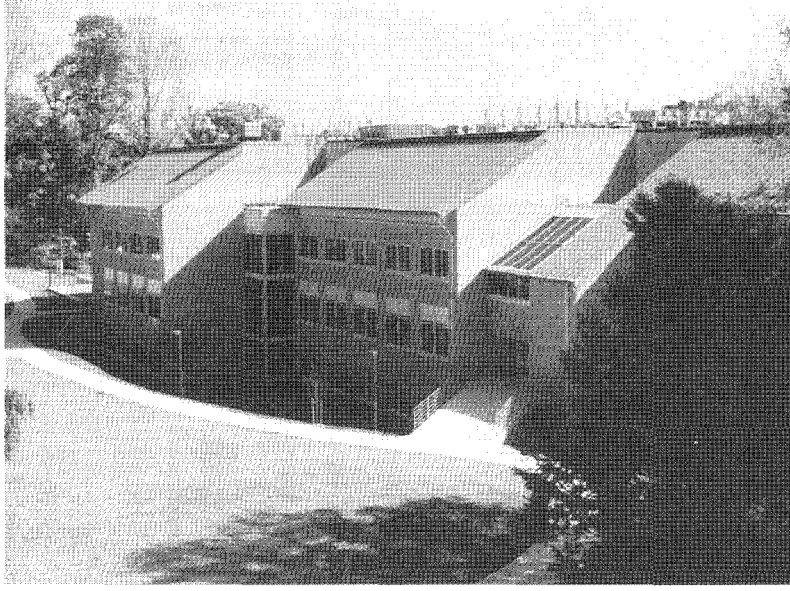
To isolate the different amino acids from a fossil shell, the inorganic material of the shell must first be removed. This is done by placing the shell in hydrochloric acid, which dissolves the minerals. The organic residue is then heated to ensure the breakup of any remaining peptide bonds.

In his early experiments at the Geophysical Lab, Hare measured the amounts and quantities of each amino acid by what is called column chromatography. The sample containing the unknown mixture of amino acids is dissolved in a solvent and then poured into the top of a column packed with material to which the amino acid molecules are able to adhere. Depending on the relative attraction of each amino acid for the solvent and the material in the column, each type of amino acid moves downward at its own rate, emerging at the bottom at different times. There, it can be collected and its quantity measured.

As time passed, Hare helped develop more-sophisticated, more-sensitive, and faster amino acid analyzers. Now, he and his colleagues use a variety of automated separation instruments; some are column chromatographs, others are gas chromatographs, where the sample's ingredients are first converted to gas, ionized to impart an electrical charge, and then allowed to land on special sensors. In one of Hare's instruments, a special fluorescent reagent reacts with each amino acid emerging from a column. The intensity of the fluorescence is measured by an instrument attached to a recorder. The recorder produces a computerized graph showing the amino acids as individual peaks (right). The concentrations of the amino acids can be determined by measuring the areas of the peaks.



drawn there by Abelson's pioneering research on amino acids in fossils. Before reading about Abelson's discovery, Hare had no idea that amino acids "would hang around so long." He decided to pursue amino acid research, and obtained a Ph.D. in geochemistry at the California Institute of Technology.



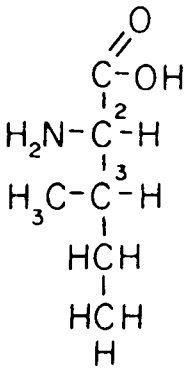
The Geophysical Laboratory and another of the Carnegie Institution's research departments—the Department of Terrestrial Magnetism (DTM)—moved into this modern building, built on DTM's campus, in 1990. The facility is located only about a mile away from the original Geophysical Laboratory, erected in 1907. Though housed on the same campus, the two departments maintain their separate identities. DTM scientists study earth, planetary, and astronomical sciences, and often collaborate with the earth scientists at the Lab.

When Hare started to work at the Laboratory he wasn't trying to develop a method to date fossils; at the time, he was interested in learning how amino acid decay differed among different mollusk species, and how the shell proteins help harden the shell. Besides, the amino acid content of a fossilized shell appeared to be too complex to use as a reliable dating tool; some of the amino acids in a shell, for example, are actually the decay products of other amino acids no longer present. How, then, was one to know which amino acids were original and which decay products?

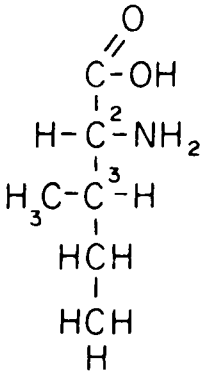
Not long after he started his experiments, however, Hare, then working with predoctoral fellow Dick Mitterer, made a discovery that was to change the direction of his research. When separating mixtures of amino acids using chromatographic columns (see box, previous page), the two noticed an amino acid present in the fossil shells that was not present in modern shells. Upon analysis, this amino acid turned out to be alloisoleucine. Looking it up in the literature, Hare learned that alloisoleucine is not normally present in unheated modern mollusk shell protein, nor in any living protein. It was listed as a heat-decay product of isoleucine, an amino acid which is present in living tissue.

Apparently, no one had ever seen alloisoleucine in fossil shells before. But Hare and Mitterer found a lot of alloisoleucine in their fossil shells, and the older the shell, the more they found. It exhibited a structural form identical to isoleucine, except for a slight change in the orientation of one of its carbon atoms (see box, next page). Though the two forms looked nearly identical, alloisoleucine and

WHAT IS EPIMERIZATION/RACEMIZATION?



isoleucine



alloisoleucine

Isoleucine and alloisoleucine have the same chemical formula, but differ in the arrangement of one of their two chiral carbon centers (designated above as C^2 and C^3). A chiral carbon is a carbon joined to four different chemical groups. Two of these groups are able to exchange under certain conditions. In amino acids, the amino group (NH_2) and the hydrogen atom can exchange when the protein chain containing the amino acid begins to break apart (generally upon heating). When this exchange process, called epimerization, occurs in isoleucine, alloisoleucine is formed. The conversion of isoleucine to alloisoleucine continues at a measurable rate over time, depending on temperature. The ratio of one to the other provides an indication of how much time has passed since decay began (i.e., since death of the organism).

When a molecule has only one chiral carbon, and two of that carbon's adjoining chemical groups exchange, the resulting compound, called a D isomer, is a mirror image of the original compound, called an L isomer. This process is called racemization. Racemization produces two optically active isomers that are mirror images of each other. In the 1960s, separating such isomers was not possible using chromatography. One had to add various enzymes to selectively "chew up" one or the other isomer. Hare says it took him weeks to do a single analysis. Today, modern amino acid analyzers can separate the two isomers of racemized samples within minutes. Still, the epimerization of isoleucine to alloisoleucine is usually the reaction of choice among scientists interested in dating older fossils.

isoleucine are geometrically asymmetric. Hare and Mitterer could thus separate—and measure—the two using standard chromatographic techniques.

The conversion from isoleucine to alloisoleucine over time is an example of a process called epimerization. Hare found that isoleucine is one of four amino acids that exhibit epimerization. Most of the other amino acids in living systems instead undergo a similar decay

process called racemization (see box). Racemization and epimerization are chemical reactions, and so must conform to chemical laws. They proceed at specific, definable rates that depend upon the nature of the molecules involved. They also depend upon various reaction conditions, most critically temperature. The speed of racemization/epimerization is related exponentially to the amount of heat supplied over time.

Hare and his colleagues used a standard mathematical equation to develop a model of isoleucine-to-alloisoleucine conversion. Their model predicted a linear relationship between the amount of alloisoleucine formed and the amount of time the reaction was allowed to proceed at a given temperature. Thus, if the investigators knew the temperature at which a fossil shell had been buried, they could come up with a pretty good estimate of that fossil's age.

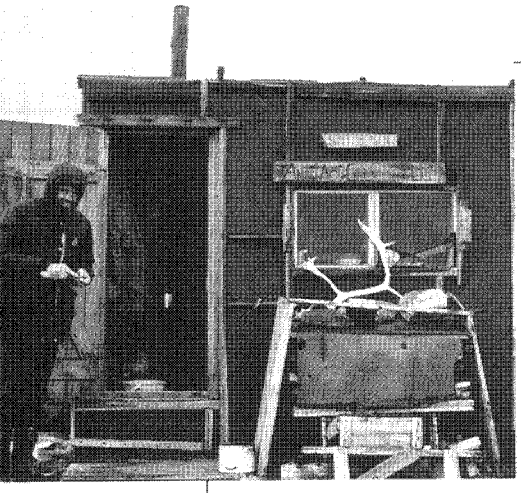
But how is one to know the temperature history of a fossil? Analyses of certain rock crystals nearby may help. But changes in these crystals provide estimates only of the maximum and minimum temperatures to which a geological outcrop has been exposed. Hare and his colleagues developed another way to calibrate temperature, based on epimerization of nearby fossils young enough to be dated by radiocarbon analysis.

The problem with this method is that temperature histories are not always straightforward within and between geological strata. There is no way to tell, for example, whether some event prior to the formation of the younger fossil may have heated the older fossil. Even a brief exposure at the Earth's surface may change a fossil's temperature profile. Because of this, Hare has cautioned many times, ages derived from epimerization and racemization data are not absolutely accurate. They can provide estimates only.

Hare's discovery of amino acid epimerization and racemization in fossils—he calls the discovery "serendipitous"—was to send him down a path that would eventually intersect with the dating needs of anthropologists. For many years, however, the use of AAR (a term encompassing both reactions) was limited to problems in the geological sciences. In most cases, the material analyzed was fossil mollusk shell.

Then, during the 1970s, groups elsewhere began to apply the technique to fossil bone. One group used racemization to date fossil human bones unearthed in California. The results were sensational. The scientists dated some of the bones at 70,000 years—tens of thousands of years older than any other human bones found in North America. Did this mean that humans had inhabited North America far longer than anyone had thought? Or—as Ed Hare claimed—was something wrong with the interpretation of the data?

Hare maintained that bone was not as suitable a material for



A PORTABLE AMINO ACID ANALYZER

In the 1970s, Ed Hare was not yet involved in anthropological applications of AAR, other than to decry the use of AAR on bone (see text). He was, however, very interested in using AAR to date geological formations. To this end, he and Gifford Miller developed a portable amino acid analyzer that they carried on various geological expeditions. At left, Hare stands outside of an old trapper's hut in West Spitsbergen, well above the Arctic Circle, where he and Miller set up the machine (right) to resolve a long-standing dilemma. Ancient beaches once existed on Spitsbergen's high and dry landforms. Researchers who dated the marine fossil deposits by radiocarbon dating estimated the age of the deposits at about 14,000 years. According to geological evidence, however, Spitsbergen was covered with ice, not water, 14,000 years ago. Something was wrong. By measuring racemization in fossil mollusk shells with their portable analyzer, Hare and Miller determined that the Spitsbergen deposits were actually laid down in two separate events—one occurring less than 10,000 years ago, the other more than 35,000 years ago. Radiocarbon dating was able to measure only an average age of the fossils.

amino acid analysis as shell. The problem is leaching. While most mollusk shell is composed of alternating layers of protein and calcium carbonate in a very tight matrix, protein in bone exists in a much looser orientation. If enough water is present in a bone's burial site, the proteins and amino acids can readily leach out. At the same time, amino acids from other sources can seep inside, contaminating the sample. And, as Hare says, "in nature, water or water vapor is almost always present."

In time, Hare was proved right; by the early 1980s, AAR had largely departed from the anthropologist's domain.

The Eggshell Project

It was not particularly surprising that anthropologists had become so interested in AAR in the first place. It has been somewhat of a tradition in anthropology, especially in the U.S. and increasingly so since the 1960s, to borrow from other disciplines. Most anthro-

pologists who practice archaeology* today are not interested so much in finding beautiful treasures (the "Indiana Jones style" of doing archaeology). Rather, they are interested in knowing how people used to live. "We want to know the social history, the everyday life of prehistoric peoples," explains Julie Kokis, a graduate student of Alison Brooks at George Washington University. "If you're interested in those kinds of things and not just beautiful objects—if you really want to find out what people did—then you have to look at animal remains, at objects associated with people, as well as at the site environment. Archaeologists can't do this by themselves. They need other experts—geologists, chemists, biologists, geographers, ecologists—to help them evaluate and interpret the data."

Alison Brooks, top; Julie Kokis, below.

Kokis, who claims she is "interested in everything" and gravitated to anthropology because of its broad appeal, has almost finished her graduate studies. As part of her Ph.D. thesis, she is spending time at the Geophysical Laboratory, learning the chemical procedures needed to date ostrich eggshells.

The project got started in 1982 when Alison Brooks brought her anthropology class to the Geophysical Laboratory. The visit was part of a series of visits—all in the spirit of "borrowing"—that Brooks planned to laboratories around the Washington area. Her visit to Ed Hare's lab at the Geophysical Laboratory was actually an afterthought, suggested by her husband, then program director of the anthropology section at the National Science Foundation. Hare suggested to Brooks that she bring with her something she was interested in dating. Brooks remembers being puzzled. The archaeological sites she'd been working on in Africa were 40,000–80,000 years old. There was nothing organic left. The proteins in the fossil bones were long since depleted. Hare pressed her. "Are you sure?" he asked. She thought a moment. She did have a few fragments of ostrich eggshell, which she'd gathered on a recent collecting trip, but she had read in the scientific literature that ostrich eggshells lost all their organic material within the first 5,000 years. But she brought the fragments along anyway. It was all she had.

*Anthropology is widely defined as the study of humans. Archaeology—the study of human history through fossils and artifacts—is one of four subfields. The others are linguistics (the study of language), cultural anthropology (the study of human culture), and human biology and evolution.

Alison Brooks arranges her collection of modern ostrich eggshell artifacts, including water containers and jewelry, in her office at the Smithsonian Institution's Natural History Museum. She has found beads and fragments of water containers as old as 40,000 years, indicating a long history of ostrich eggshell use.

Ostrich eggshells are common in certain archaeological sites in the drier areas of Africa and Asia. Often, they are the only identifiable animal fossils at these sites. Presumably, prehistoric peoples ate the ostrich eggs and then used the shells as water containers or to make decorative beads. Despite the ubiquity of ostrich eggshells, however, archaeologists have not considered them to be very important. "The only interesting thing about them was that they showed there were ostriches in the area, and people were eating the eggs," explains Brooks. "There was no need to save them."

While Brooks and her class were visting the Laboratory, Hare dissolved the ostrich shell fragments in some acid and tested for the presence of alloisoleucine. "Lo and behold, it was there," remembers Brooks. Over the next few months, Brooks and some of her students visited the Laboratory as much as they could, bringing along more samples of ostrich eggshell. Hare provided the experimental expertise in analyzing the samples; Brooks and her colleagues provided the anthropological questions. Collaborating with Giff Miller by mail and telephone from his laboratory at the University of Colorado, Boulder, the group found not only that ostrich eggshell contained alloisoleucine and other amino acids, but that the material seemed to be even more suitable for epimerization dating than mollusk shells. In experiments on modern ostrich shells (obtained first from the National Zoo and later from farms advertised in ostrich newsletters), they found the shells to be extremely resistant to decay. After keeping the shells in a constantly replenished water bath at 105°C for 70 hours—an experiment simulating leaching—they found that the eggshell retained 99% of its stable amino acid content. (Under similar conditions,

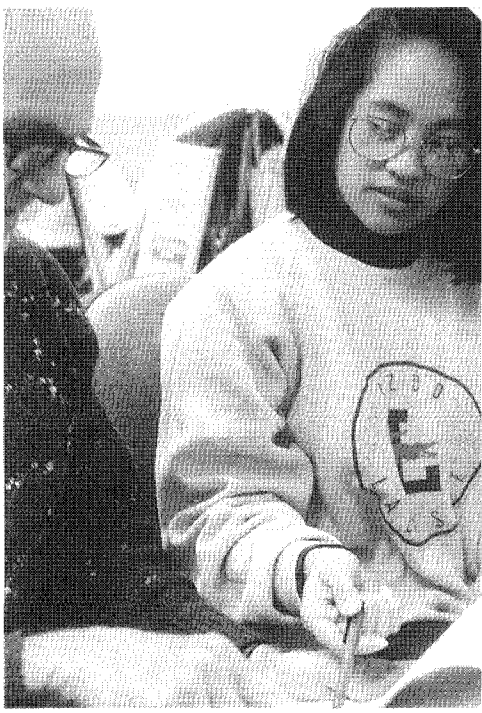
An ostrich at the National Zoo i Washington, D.C.—source of modern eggshell for comparative analyses. (©1992 by the Smithsonian Institution.)

mollusk shell retains 40% of its stable amino acids, and bone less than 5%.)

What did this mean? Writing in *Science* magazine in an April 1990 issue, Brooks, Hare, and their colleagues noted that ostrich eggshell "more nearly approximates a closed system than any other organic material yet recovered from archaeological sites." Where temperature history is known or derived from other data, epimerization of isoleucine to alloisoleucine can be used to estimate eggshell age, and hence the age of human fossils with which the eggshells are associated. At normal temperatures in the tropics and subtropics, the technique promised to provide accurate ages of ostrich eggshells up to 200,000 years old. In colder climates, where epimerization and racemization proceed at slower rates, the technique should be useful, the authors maintained, on material that is up to one million years of age.

In using ostrich eggshells to date hominid sites, however, Hare and his colleagues soon ran into a serious obstacle. It appeared that some of the ostrich eggshell samples they were using in their analyses had been heated, presumably in cave campfires. If so, heating would have changed the eggshells' temperature-sensitive epimerization profile.

While it was obvious that a few of the fossil eggshell samples had been heated (they were charred), it was less obvious that others had been. "Some of the shells might have been placed near a fire for a couple of weeks at, say, 200°C," says Brooks. "And we couldn't see any visible difference between those shells and the shells that were unheated."



Hare goes over some data with Carnegie research intern Karen Durana, a student at nearby Columbia Union College, who performed many of the fire-simulated heating experiments at the Laboratory.

To study the problem, Ed Hare and his colleagues, including college student Karen Durana, an intern supported by the National Science Foundation, conducted heating experiments on modern eggshell. They wanted to learn what happens to eggshells when they are heated to the very high temperatures characteristic of campfires. And so they used no water. "Heating with water duplicates what nature does," explains Hare. "There's water everywhere—not a single place on Earth is without water vapor." But in a campfire, the moisture is so dramatically lessened that it is almost as if it were absent.

Hare says that the chemical differences between the burned and the unburned samples are startling. The burned shells, for example,

contain a great deal of ammonia (in the form of the ion NH_4+); unburned shells do not. "When an amino acid's amino group (NH_2) is destroyed by burning," Hare explains, "ammonia is formed. In some shells, those heated dry to about 360°C—comparable to a good campfire—ammonia is all that's left." The campfire-heated shells also contain other clues, such as amines, which represent an intermediate stage after the carboxyl group is stripped off the molecule but before ammonia forms. The more amines a shell contains, the greater the temperature to which it has been heated.

Hare and Brooks are pleased that the campfire obstacle seems to be overcome. "We think we're pretty close to being able to say, 'Aha, this sample was burned, so we're not going to use it to calculate our data and determine ages,'" Hare says. In the meantime, they have proceeded with the eggshell project on two fronts. They are beginning to ask if other bird eggshells are appropriate for amino acid analysis. Ostriches can't be used to date hominid sites in Europe, for example, because no ostriches lived in Europe. But owls did. And so the group is studying owl eggshells, developing techniques using modern samples. "The modern samples are always done first,"

Interested in questions of human origins long before becoming involved with the ostrich eggshell study, Alison Brooks has traveled to Africa twelve times since 1968. Photos here were taken during her most recent trip, to Zaire, in 1990. Top left: with her excavation team at Katanda, a site where she and her husband, John Yellen, have found what may be the earliest complex bone tools ever made. Bottom left: with her husband after a day of digging. Above: working in the field lab with two assistants. (Photo credits: A. S. Brooks)

says Julie Kokis, who is running many of the experiments. "We have to be careful. We have so little of the fossil owl eggshells, and what we have is so much thinner and more delicate than the ostrich eggshells that we don't want to analyze them before we establish the proper procedures."

In another effort, Hare, Brooks, Miller, and their colleagues have applied epimerization techniques to date ostrich eggshells from some southern African cave sites where anatomically modern human skeletal remains have been found. Cave sites offer excellent samples for this analysis because temperature fluctuations tend to be minimal and exposure to the surface unlikely.

Gifford Miller, above, was a postdoctoral fellow in Ed Hare's lab in 1974–1975. He now runs the Center for Geochronological Research at the University of Colorado, where researchers work mainly on problems of Quaternary Arctic climate change using racemization in mollusk shells. Miller also studies aspects of human evolution and migration. In an effort independent of the Carnegie group, he spent much of 1992 on sabbatical at the Australian National University, Canberra, studying racemization in eggshells of extinct ratites. The work suggests that humans were established in Australia at least 50,000 years ago, and that, at about the same time, the marsupial population was drastically reduced, possibly because of forest burning by the earliest human immigrants. (Photo credit: Ken Abbott)

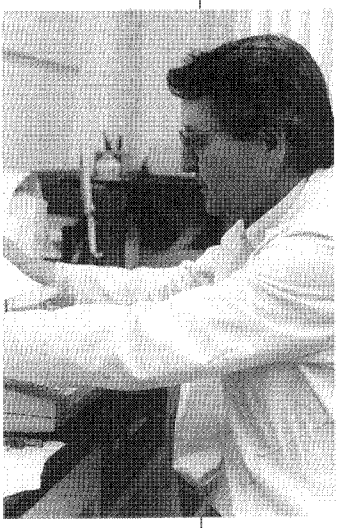
Previous ages for the southern African human fossils, based on a wide variety of techniques, ranged from 50,000 to 130,000 years. The new ostrich eggshell epimerization dates obtained by the Carnegie group suggest that anatomically modern humans were well established in southern Africa by about 100,000 years ago. Their data lends evidence to the hypothesis that modern humans did, indeed, originate in Africa.

Although these results are exciting, the use of epimerization and racemization in anthropology, like TL and ESR, has a long way to go. Brooks says she has had positive response from many of her colleagues (who, she says, are now saving their ostrich eggshells), but others are not yet convinced. In a 1990 interview for *Science* magazine, Hare said they'd had trouble convincing people that AAR works in mollusks and ostrich eggshell "because all [these people] can picture is that nonsense with bones."

Many scientists are not sure that AAR, or any of the new dating techniques borrowed from the natural sciences, have been perfected enough to be reliable. And, indeed, there is conflicting evidence. In the southern African cave work, for example, Hare, Brooks, and Miller's epimerization data are consistent with TL data but conflict with ESR ages by as much as 40,000 years.

COLLABORATIONS

Hare's reputation, along with his collection of advanced instrumentation, has for years attracted a variety of students, postdoctoral fellows, and visiting investigators to the Geophysical Laboratory. These individuals spend anywhere from several months to several years in collaboration with Hare.



A current collaborator is David von Endt, left, from the Smithsonian Institution. von Endt is concerned about the deterioration of what he calls our "biodiversity storehouse"—natural history specimens in museums. Many of these specimens, he says, are our only samples of extinct organisms. He is beginning his study by first examining the fluids—the alcohol—in which the specimens are preserved. He measures the fatty acids, amino acids, and carbohydrates that seep into the fluids, using a specially adapted gas chromatograph instrument in Hare's lab.

Ed Hare has for many years enjoyed a close relationship with scientists at the Smithsonian Institution. In one interesting case several years ago, he was approached by a Smithsonian scientist who had received in the mail a piece of human skull from Denmark. The skull, found in a peat bog, was in such good shape that the police suspected foul play. But they had no accurate way to date the skull. Applying racemic techniques to the sample, Hare found the skull to be several hundred years old. Radiocarbon analysis later confirmed the age. The case was closed.

Straightening out the inconsistencies, however, is all part of the scientific process. Anthropologists have learned they cannot rely on a single piece of evidence alone, or on a single dating technique.This is why, Kokis says, "in dating sites, the more separate lines of evidence that can be brought to bear, the more weight one can give to the answers."

Brooks, for one, is convinced that amino acid dating methods will prove reliable. "In the time frame that we're dealing with—beyond radiocarbon dating—amino acid racemization [of ostrich eggshell] may be one of the best techniques we've got."

Meanwhile, Ed Hare, though still involved with the eggshell project, is beginning to return to his overriding passion—to understand in even greater detail the complexities of epimerization, racemization, and other reactions, and to learn how these and related processes can provide further insight not only into human history but into the much longer history of the Earth itself.

Isotopes as Dietary Tracers:

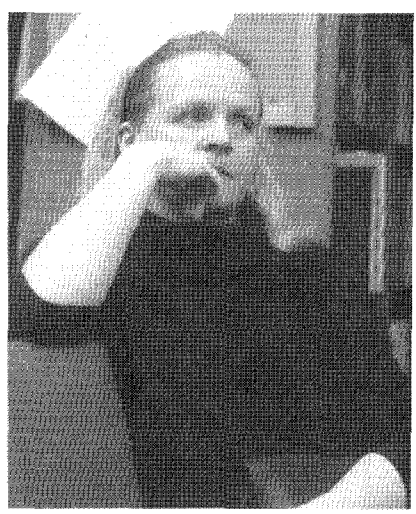
Studying Nursing Patterns Among Prehistoric Peoples

About a thousand years ago, by 900 A. D., maize, or corn, had become a dietary staple among people living on the North American continent. Though humans had begun to cultivate plants thousands of years earlier, no other crops assumed the dietary importance that maize did; even while tribes relied on bison or other game, the introduction of maize agriculture brought great changes. By growing their own grain, a tribe could put up foods for the winter months, when there were no wild plants to gather and game was scarce. The inconsistencies of the food supply could thus be abated.

Along with the development of agriculture came a large spurt in population growth. The reasons for this are unclear. One fairly well-established hypothesis among anthropologists holds that mothers in more-primitive hunting-and-gathering societies breastfed their infants two or three years longer than mothers in agricultural populations because there often was no food available to wean them to. By doing so, the hypothesis suggests, they may have delayed the time intervals between births and kept the population low.*

In 1989, a pair of researchers from the Geophysical Laboratory and the Smithsonian Institution provided evidence that the hypothesis is wrong. Carnegie's Marilyn Fogel and the Smithsonian's Noreen Tuross found that women in hunting-and-gathering societies appear to have nursed their children for the same amount of time as those in agriculture-based societies.

That these two scientists could study breastfeeding patterns among peoples who died thousands of years ago seems almost impossible. Indeed, when Fogel and Tuross applied for a grant from the National Science Foundation to study nursing strategies in archaeological human populations, they were turned down with an unambiguous no. "It can't be done," wrote the reviewers. But



Marilyn Fogel, above, and Noreen Tuross

*A nursing mother is generally able to conceive about six months after the birth of a child. To do so, however, her body must have a certain fat content. Nursing women in hunting-and-gathering societies, the anthropologists who espouse this theory argue, may have had trouble keeping their weight up because of the demands of nursing coupled with the rigors of an inconsistent food supply.

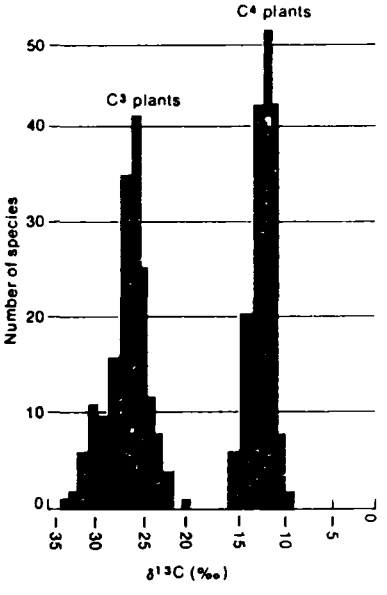
Fogel and Tuross went ahead anyway, managing, with the tools of modern isotopic analysis and a few inspired experiments, to turn a favored anthropological hypothesis on its head.

You Are What You Eat

Fogel and Tuross arrived at their conclusion after a year of Wednesday collaborations at the Geophysical Laboratory. The two scientists measured stable isotopes (see box, next page) preserved in two collections of fossilized Indian bones: one representing a hunting-and-gathering society, the other an agriculture-based society. The isotopes told them something about what the individuals in each group had eaten during the last few years of their lives. This is because the isotopes mirrored the isotopic signatures present in their foods, including the breastmilk consumed by babies who died very young.

Different foods have characteristically different isotope ratios because of the way those isotopes, having different mass, "fractionate," or partition, during the various chemical reactions that characterize metabolism. For carbon isotopes, photosynthesis—at the base of the food chain—is the most critical reaction. The isotopes fractionate in one of two main ways depending on how a plant initially processes its incoming carbon dioxide. Most terrestrial plants in North America and Europe, including trees, shrubs, and temperate grasses, use what is called the C_3 pathway, where incoming carbon dioxide is initially converted to a compound having three carbon atoms. Important C_3 crop plants are rice, wheat, and barley.

Another set of plants, including tropical grasses and other plants



Carbon isotope ratios of several hundred species of plants, plotted in a histogram, fall into two separate clusters, illustrating the distinction between C_3 and C_4 plants. The measurements, in parts per thousand, represent the ratio between the ^{13}C and ^{12}C as compared to a standard having a ^{13}C of zero.

Corn, like all C_4 plants, has a relatively high ratio of ^{13}C to ^{12}C. The carbon isotope ratios of modern North Americans are similar to that of corn, mirroring a diet rich with meat from corn-fed animals.

adapted to the hot sun, use the C_4 pathway, where carbon dioxide is initially converted to a four-carbon product. Among these plants are maize and sugar cane.

Both pathways—C_3 and C_4—yield a distinctive ratio of ^{13}C to ^{12}C, a ratio which shows up in every tissue of the plant. That ratio is transferred to the organism that eats the plant, and is maintained faithfully as one goes up the food chain. For example, an animal that eats grass has the same carbon isotope signature as the grass. So, too, by and large, does the human being who eats the animal. Because of this, Fogel, Tuross, and others are able, through isotope analysis, to learn the general plant source of an individual's food. Isotope analysis cannot, however, tell exactly which plants a person—or animal—has eaten. The signatures can only tell generally if that organism is predominantly a C_3 or C_4 eater.

While carbon isotopes change little from prey to predator,

WHAT IS AN ISOTOPE?

Isotopes are atoms having the same atomic number but different atomic mass. Some, like ^{14}C, are unstable because they decay with time, releasing radioactive particles. Others, like ^{12}C and ^{13}C, are stable and do not decay.

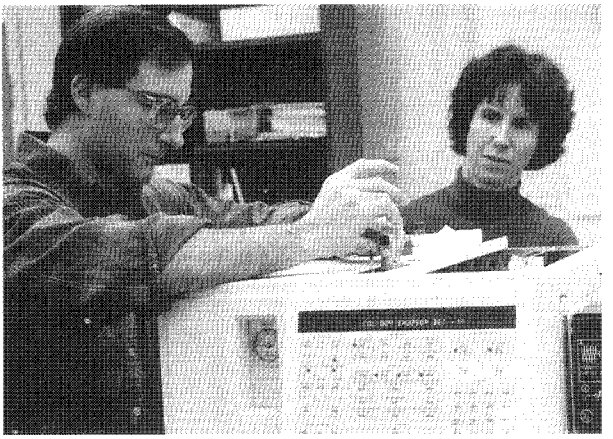
Carbon and nitrogen are the two elements whose stable isotopes are most commonly used in food-tracer studies. Carbon exists predominantly as ^{12}C with a trace of ^{13}C. Nitrogen occurs as a mixture mostly of ^{14}N and a trace of the heavier isotope ^{15}N. The proportion of two isotopes in a sample can be measured using a highly sensitive instrument called a mass spectrometer. The ratios are then compared to a standard and expressed in parts per thousand, using the symbol ‰.

Though isotopic analysis has been used by geochemists as a dating tool for many years, it wasn't until the mid-1970s that scientists learned that stable isotopes could tell something about diet. Anthropologists began collaborating with geochemists to learn how to measure signatures of stable carbon and nitrogen isotopes in fossil bones. They began developing standards for the techniques; they learned how the fractionation process differs among various tissues during metabolism, and how to account for these differences in analyzing data. Eventually, they were able to trace the introduction of maize into various parts of North America. (Prior to its introduction, C_4 plants were rare in the human diet.)

Using a Mass Spectrometer

A mass spectrometer separates the isotopes of a given element by weight, and measures their concentrations. The isotopes are introduced in gaseous form after being combusted in a sealed tube within a special furnace. Carbon isotopes are introduced as CO_2, nitrogen isotopes as N_2. The electrically charged gas ions are accelerated down a flight tube, where they are directed past a magnet. The magnet bends their trajectories. The lighter ions bend more than the heavy ones and are captured at a different detector. The concentrations are measured electronically and displayed on a computer screen.

The Geophysical Laboratory's new-generation mass spectrometer, shown below being operated by Fogel and postdoctoral fellow Jeff Silfer, is at least an order-of-magnitude more sensitive than older machines. It is also much faster; it contains its own chromatographic system, for example, which separates different compounds and subsequently measures each component's isotopes automatically in a couple of hours. Previously, this process, which required an elaborate ion-exchange system, took weeks.



nitrogen isotopes are another matter. For some unknown reason, nitrogen becomes increasingly enriched in the heavier isotope, ^{15}N, as one proceeds up the food chain. It is enriched by about 3 parts per thousand (3‰) with each step. By measuring the ratio of ^{15}N to ^{14}N, then, one can determine the general position of an animal in the chain.

Isotope signatures can be measured in a variety of animal tissues, such as skin, hair, teeth, or bone collagen. Collagen, the organic part of bone which contains most of the bone's proteins, is the most common material used with fossils. It survives for about 10,000 years in warm climates, and up to 100,000 years in colder climates. At the time of an organism's death, the isotope signatures in collagen are "frozen" in place; they do not change over time, as amino acids do. (Leaching does not seem to be a problem, as long as one uses bones with well-preserved collagen.) The signatures thus mirror the isotope ratios—and food sources—in the last years of an individual's life.

When she came to the Geophysical Laboratory, Marilyn Fogel was not generally interested in paleonutrition, or "dietary detective," studies. She was more intrigued by what stable isotopes can tell

about the nature of Earth's early organic history. Such questions remain foremost in her research program, but in 1986 she also began to be intrigued by the human past. At that time, she began collaborating with Noreen Tuross, then a visiting investigator at the Laboratory and now at the Smithsonian Institution's Department of Analytical Conservation. Tuross first became acquainted with the Geophysical Laboratory in 1976. She came to study amino acids in bones with Ed Hare while working on her master's degree. She went on to get a Ph.D. in neuroscience from Brown University, but she never lost her interest in the molecules preserved in bones. She now calls herself a bone biochemist.

Marilyn Fogel obtained her Ph.D. at the Marine Science Institute of the University of Texas, and then spent two years of fellowship at the Geophysical Laboratory before being appointed a staff member in 1979. One of her first ventures at the Lab was to gather algae and bacteria from the hot springs of Yellowstone National Park. Above, she collects sulfur-oxidizing bacteria from an acidic, boiling hot spring. By studying the distinctive carbon, nitrogen, and sulfur isotopic signatures of these organisms, she hoped to learn the nature of the microorganisms that first populated the Earth.

Fogel and Tuross's foray into anthropology began with bones from an 8,000-year-old Florida grave site—the Windover site—discovered in the early 1980s. The remains, buried in a peat bog, were so well preserved that DNA was found intact in the brains. Fogel and Tuross were more interested in the bones' fossilized collagen.

Working at the Geophysical Laboratory, the two dissolved the bones' inorganic parts and isolated the collagen. They measured the collagen's carbon isotope signatures with the mass spectrometer. All the samples, they found, were enriched in ^{13}C, much like modern maize consumers. The simple interpretation is that these people ate maize. Maize, however, was not introduced into the region until about 900 AD. What, then, accounted for such a high percentage of ^{13}C? An isotopic analysis of animal bones collected at the site provided part of the answer. Windover was an aquatic site 8,000 years ago, and the animals had isotope ratios showing the presence of aquatic C_4 grasses. Presumably, the animals ate the grasses and the people ate the animals. Nitrogen analysis showed that the humans were omnivorous, eating both meat and some kind of succulent, most probably a common cactus.

Finding a "Nursing Signal"

While working on the Windover samples, Fogel and Tuross began to speculate whether it might be possible to use nitrogen isotopes to learn about the nursing strategies of ancient human populations. Might a breastfed infant be distinguished isotopically from its mother in a measurable way, they wondered? Because an infant, in effect, "preys" on its mother's milk, they hypothesized that the difference might correspond to a step in the food chain. If so, then its tissues should be enriched in ^{15}N by 3‰, and that enrichment should show up in the fossilized infant bones.

Their first task was to determine if their hypothesis—that an infant is one step removed from its mother in the food chain—was correct and measurable. For this, they needed a modern population. They couldn't, of course, use bones or teeth from a living baby, but they *could* use fingernails. Not only are fingernails easily obtained, they are also a good protein (i.e., nitrogen) source. As far as Fogel and Tuross knew, no one had measured the nitrogen isotope ratios of infant fingernails before. "We had no idea what to expect," Fogel says.

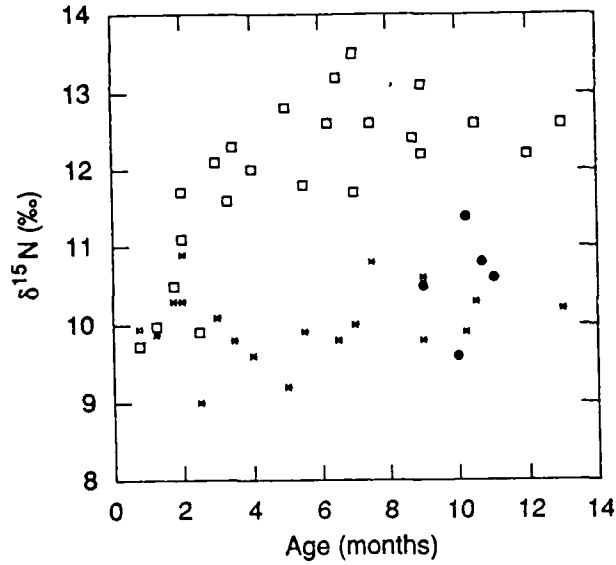
A year passed before they began experiments. In the meantime, Fogel had given birth to and was nursing her first child, Dana. The timing was fortuitous. Mother and daughter donated their first set of fingernail clippings when Dana was six weeks old. The results were disappointing. No difference showed up between Fogel and her daughter; both sets of fingernails had virtually the same isotope compositions.

Six weeks later, they tried again. They had learned in the meantime that fingernails in healthy infants require from two to three months to grow from cuticle to fingertip. Thus, when a baby is born, it has fingernails that starting growing *in utero*, when isotopically an infant is no different from its mother. Only after the birth nail is gone and replaced by new nail is isotopic variation measurable.

The results at four months were just as they expected. Dana's fingernails were enriched in the heavier nitrogen isotope by 3‰. Fogel and Tuross were ecstatic. "It's there. It's there," Fogel remembers shouting.

The two researchers began to round up additional experimental subjects: nursing mothers and their infants. Friends, professional colleagues, friends of friends—they had no trouble finding volunteers. Eventually, they had a small

Fogel and Tuross's first experimental subject: Fogel's daughte Dana Swarth, at age six months (Photo credit: Lorraine Miller)



Isotope variations in fingernail samples from 16 mother (asterisks) and infant (squares) pairs are shown in plot above. All infants were fully breastfed for at least three months, when a variety of alternate foods were introduced. Nitrogen enrichment begins in the infants at between two and three months of age, once old fingernails are replaced by new growth. Children who were totally weaned to non-human milk or milk-based formula are indicated with solid circles.

population of sixteen pairs, each of which donated fingernail clippings at irregular intervals for fourteen months. All infants were exclusively breast fed for at least three months, when a variety of alternate foods were introduced.

The results for each mother-child pair were the same: all mothers had remarkably similar ratios of ^{14}N to ^{15}N, while their nursing babies were, on average, enriched in ^{15}N by a statistically significant +2.4‰. As the babies were weaned, the enrichment declined (see graph, above). To Fogel and Tuross, here was evidence that a "nursing signal" does in fact exist.

Their next question was whether this signal would also exist in bones of archaic populations. Because they were already familiar with the Windover site, they started there, first looking at rib bones. "We had samples from children anywhere from zero to four years old," says Fogel. "We found some enriched and some not. We struggled and sweated to get a pattern, but the ages weren't exact enough." It wasn't until several months later, when Smithsonian anthropologist Douglas Owsley joined the team, that their study could proceed. Using infants' teeth and bone sizes, Owsley was able to determine age at death to within one or two weeks.

Owsley provided Fogel and Tuross with two sets of prehistoric human bones. One set came from a site in the Tennessee Valley dated from 2000 B.C., nearly 3,000 years before maize became a dietary staple in North America. These people were hunters and gatherers, moving from place to place in search of food. Another set of bones came from a site in South Dakota, and were dated at about 1600 A.D. These bones represented a people who practiced maize horticulture in addition to hunting.

Douglas Owsley of the Smithsonian was able to determine precisely the age at death of prehistoric infants. Above, he excavates a 17th century burial near Yorktown, Virginia. (Photo credit: Chip Clark)

In their nitrogen-isotope analysis of infant and child bones in both archaeological populations, Fogel and Tuross found nearly identical patterns: ^{15}N enrichment was present until the infants were about eighteen months old. This meant that babies were weaned, or at least given alternate food sources, when they were a year and a half old.

Tuross says they were surprised at the result. They had expected to find that women in the hunting-and-gathering population nursed their babies longer than those in the later, agriculture-based population, as many anthropologists assumed. Speculating about their results, Tuross says that mothers in agricultural societies *could* have introduced maize gruel earlier, and the babies would have eaten it. But they didn't. "The data show that the American Indians, at least in this population, knew *exactly* what they were doing. They knew if they fed maize pap to their babies earlier, the babies would not thrive." (Maize, unlike mother's milk, is not rich in protein. In our modern society, we introduce cow's milk, a substitute protein source, at an early age. But the American Indians had no cows or other substitute milk sources.)

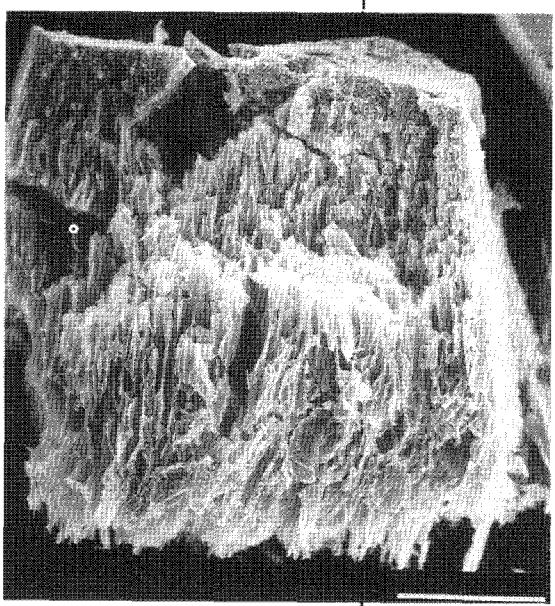
If Fogel and Tuross's results hold true for other archaic populations, both hunting-and-gathering and agriculture-based, then it will rule out the possibility that birth spacing decreased in agricultural societies because women reduced the duration of breastfeeding.

Fogel and Tuross, however, will not be pursuing such studies in the near future. Fogel has returned to more-basic questions about organic decay. She is, for example, using nitrogen-isotope analysis to study the effects of acid rain on coastal plant communities. She is also studying the decomposition of plants immediately after they die. Why, she wants to know, is there an increase in nitrogen in a plant eighteen months after its death? It is a question she hopes will shed light on a larger problem—how

kerogen, the organic precursor of gas and oil and the dominant organic material on the Earth, is formed. (Fogel gave birth to her second child, Evan, in June 1991.)

Tuross, meanwhile, is interested in taking isotopic monitors farther in time, "where less is known and where molecules have more to contribute." Accordingly, she has just begun a project, partly in collaboration with Fogel, looking at the mineral apatite in ancient shells of the horseshoe crab. Though apatite is inorganic, it does contain isotopic signatures. But its use—as the following section makes clear—is controversial.

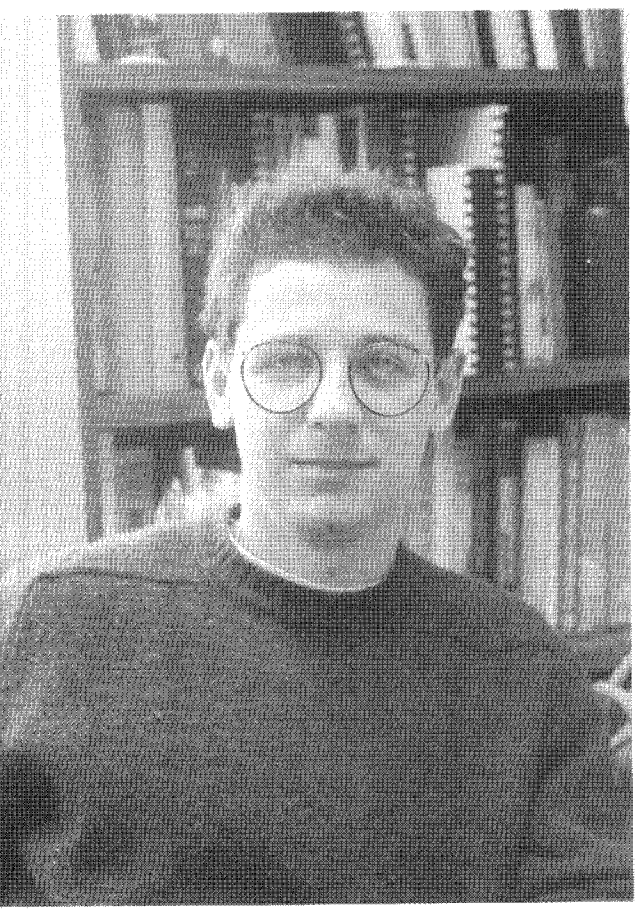
In research that links the two earlier sections in this essay (ostrich eggshells as dating tools and stable isotopes in paleodietary reconstruction), Carnegie predoctoral fellow Beverly Johnson (below left) is currently exploring with Marilyn Fogel the usefulness of stable isotopes in fossilized ostrich eggshell as a tool for reconstructing the diets of the ostriches who laid the eggs thousands of years ago. They hope this information will contribute to a better understanding of the environment, including climate and available food supply, in which early modern humans lived.



Johnson, a doctoral student of Gifford Miller's at the University of Colorado, says that the organic constituents of ostrich eggshell are well preserved by the mineral matrix, and provide a new and currently unexplored medium for paleodietary studies. Scanning electron micrograph (right) shows organic matrix of modern ostrich eggshell. Bar scale at bottom is 1 mm in length; inside of eggshell is at top. (Photo credit: Claire Rutiser, Conservation Analytical Laboratory, Smithsonian Institution.)

Studying How Bones Decay

Paul Koch is a postdoctoral fellow at the Geophysical Laboratory who is particularly interested in apatite. Apatite, short for hydroxyapatite [$Ca_5(PO_4,CO_3)_3(OH,F,Cl)$], is the mineral in bones and teeth. Like collagen, apatite contains stable carbon isotopes, which offer a potentially valuable tool for assessing the diets of long-dead organisms. Apatite is especially important because it lasts much longer than collagen, up to hundreds of millions of years. It is also valuable because it contains isotopes of oxygen (^{16}O and ^{18}O). Apatite is, in fact, the only source of stable oxygen isotopes in fossilized mammals.

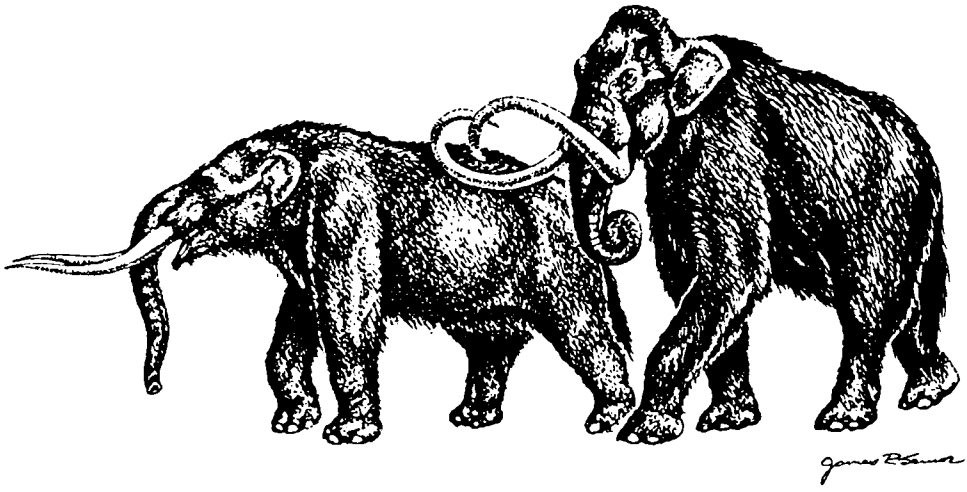


Paul Koch in his office at the Geophysical Laboratory. Koch decided to pursue a career in paleontology in his last year as an undergraduate at the University of Rochester. He also liked English and biology, and graduated with a double major in English and geology.

The use of apatite in dietary reconstruction studies, however, is controversial. Although apatite in teeth seems to maintain its integrity over time, apatite in bone does not. It can exchange chemical elements with those in groundwater, causing alterations in the isotope ratios. Some scientists believe this exchange can be monitored and corrected for. Many others do not.

Koch first became interested in apatite while doing graduate work at the University of Michigan. He decided early in his studies at Michigan to focus on paleoecology—the interaction of ancient organisms and their environments—by using the tools of isotope geochemistry. The traditional method of doing paleontology is to look at the shape of bones and teeth. Isotope geochemistry offered a new approach. Koch was intrigued that "you could dissect the molecules of an animal and gain some knowledge about its biology."

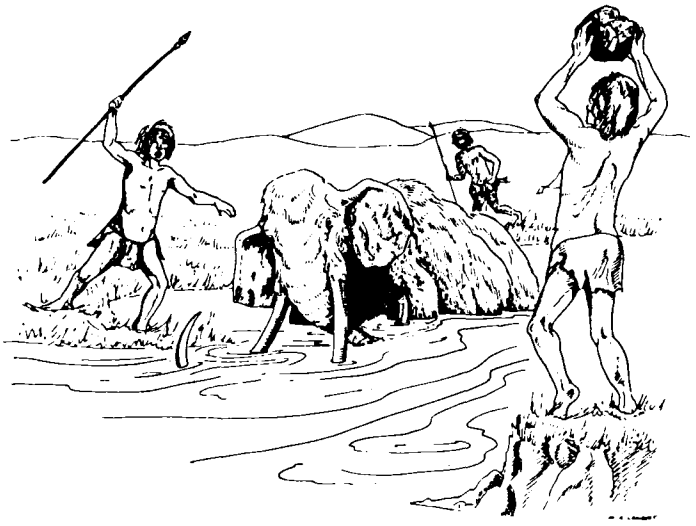
In 1983, with Dan Fisher, his advisor at Michigan, he began studying apatite in the growth rings of fossilized tusks from 11,000-year-old mastodons and mammoths. (Like trees,



The mastodon, left, and mammoth, right, were distantly related cousins of modern elephants. Mammoths had higher foreheads, more highly curved tusks, and flatter teeth. They also had longer legs and thinner bones, which probably meant they were more mobile than the more muscular mastodons. Both beasts were prominent in the Pleistocene (an era that began some 1.8 million years ago and ended 11,000 years ago), and both became extinct by the end of the era. (From *Pleistocene Mammals of North America,* by B. Kurtén and E. Anderson, Columbia University Press, 1980.)

tusks contain annual rings that reflect age and seasonality.) Apatite was particularly appealing to Koch and Fisher because it contained oxygen. A living animal reflects the oxygen signatures present in its local drinking supply. Those signatures, in turn, reflect the temperature of the local climate. While ^{16}O is always the predominant isotope, there is slightly more ^{18}O present in warm climates than in cold. Because of this, scientists can measure the ratio of ^{16}O to ^{18}O in fossilized apatite and use the data to reconstruct the temperature histories of ancient environments.

Oxygen isotopes can also help scientists determine the season of an animal's death. Mammoths and mastodons are particularly well-suited to such analysis because the annual rings in their tusks reflect seasonal changes. (There is slightly more ^{18}O present in animals that die in the warm summer months.) Koch and Fisher were interested in learning whether the animals were intentionally slaughtered by human beings or merely scavenged after dying of natural causes. They had a sample of tusks from animals they had reason to believe were butchered and another sample from animals that were not. They reasoned that if the butchered animals had been scavenged and not hunted then they should have died at the time of year when the



Could hunting have caused the spectacular extinctions of the mastodons, mammoths, and other large mammals 11,000 years ago? Or were other, natural causes responsible? Koch hopes that an isotopic analysis of diet may provide some answers. (From *Pleistocene Mammals of North America*, by B. Kurtén and E. Anderson, Columbia University Press, 1980.)

animals naturally died—in the late winter or early spring. But if the butchered animals died at the hand of hunters, they might have died at other times of year as well. By revealing that butchered and nonbutchered animals died in different seasons, the oxygen isotope results indicated that humans did indeed hunt the animals.

Though interesting, the results of this study pointed out the uncertainties of using apatite. Koch says that even though they used only well-preserved fossil tusks, they still found that some tusks were contaminated, that is, isotopes in the tusks had exchanged over time with isotopes in the ground-water.

Despite these uncertainties, Koch was not ready to dismiss apatite entirely. Soon after completing the butchering study, he began a new study, using isotopes in both apatite and collagen, to assess the nutritional status of mammoths and mastodons. About 11,000 years ago, at the end of the Pleistocene era, mammoths and mastodons, as well as many other large mammals, became suddenly extinct. Koch wondered if the extinctions could have been caused by human hunting alone or if there were other, more natural explanations. This question—just what killed the Pleistocene giants—is one that has been hotly debated for over a hundred years.

When he first started his studies at Michigan, Koch was all but convinced that the Pleistocene extinctions were caused chiefly by humans, at least in North America, where some thirty species of large mammals were extinguished in the span of several thousand years—

an eyeblink in geological time. Humans arrived in North America at the end of the Pleistocene with fairly lethal weapons and tools. Their hunting skills were well enough developed, Koch believed, to have hunted the great Pleistocene beasts to extinction.

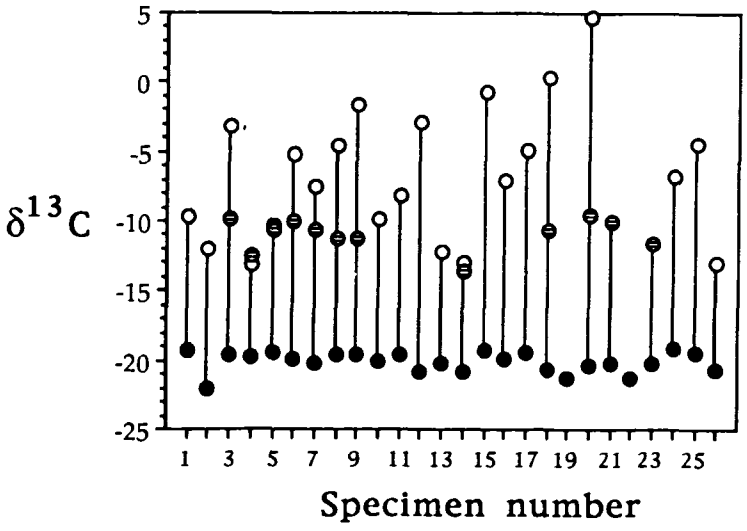
Though others have advocated such an "overkill" scenario, evidence has been slim. Koch and Fisher's oxygen isotope study at the University of Michigan provided a convincing argument that humans did indeed hunt and not merely scavenge mastodons. But Koch was—and is still—skeptical about drawing global conclusions from such a small study. "I do believe that mastodons were hunted, not scavenged," he says, "but did humans kill enough beasts to have an effect on extinction? That's not the same question."

Koch was curious about arguments that natural events, especially changes in climate, caused or at least contributed to the extinctions. The Pleistocene was an era characterized by the waxing and waning of great glaciers. During their movements south, the glaciers held much of the northern hemisphere in a tight grip of cold. Those who favor natural causes for extinction argue that the last retreat of these glaciers, at the end of the Pleistocene, was more rapid than any previously experienced, and that this retreat led to extreme seasonal changes in climate.

As a result, suggests one group of scientists, there occurred a dramatic shift in plant distribution. Plants that today live nowhere near each other lived side-by-side during the Pleistocene. Animals became dependent on the variety of different foods. When the climate changed and plant distribution became more like what it is today, according to the hypothesis, the animals couldn't find the plants they needed and starved to death.

Koch was intrigued by this hypothesis. He proposed testing it by measuring isotopes in the bones and teeth of mammoths and mastodons, and then comparing those isotope values with isotopes in the bones of the modern counterparts to mammoths and mastodons—elephants. Elephants are able to eat almost any kind of plant, both C_3 trees and C_4 grasses. Koch reasoned that if the isotopes in mammoths and mastodons showed that they, too, were able to eat virtually anything, then there would be no good reason to believe that at least these animals couldn't adapt to a changing landscape. The starvation hypothesis would be flawed.

In this study, as in the butchering study, Koch used only well-preserved specimens of bones and tusks (those showing no evidence of alteration or decay). Because of this, he suspected that the carbon isotope values for the apatite might reflect those in the more-reliable collagen. He was wrong. In his first analyses, the apatite carbon values were, as he says, "all over the place," while those in collagen followed a fairly straight line (see graph, next page). It was conceiv-



Carbon isotope values among apatite in bone (open circles), apatite in tooth enamel (shaded circles), and collagen in bone (dark circles) for different Pleistocene mastodon and mammoth specimens. While the collagen and tooth enamel apatite values follow relatively straight lines, the bone apatite values fluctuate widely.

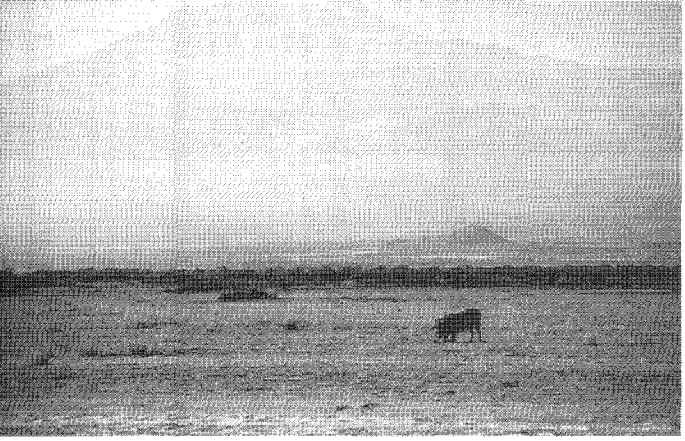
able, he acknowledges, that the apatite values could have been correct. Indeed, if he had no collagen with which to compare them, he wouldn't have known they were flawed.

The uneven results made him think hard about apatite decay. When, he wondered, did apatite become unusable for isotopic analysis? Was it right after an animal died, as its bones sat decaying on the Earth's surface, or was it later, during the subsequent long years of burial?

Koch knew that as bones lie on the Earth's surface exposed to sun and rain, they begin to bleach, disarticulate, and crack. If unburied after fifteen or twenty years, they turn completely to dust. He also knew that apatite in an animal's living tissue is present as very small crystals. As soon as an animal dies, however, the crystals begin to enlarge. Koch wondered if this physical change was related to apatite's isotopic alteration. "Given the ambiguity in my data," he says, "I figured I should test it. I should see how bones fall apart." As far as he knew, no one had studied isotope changes in apatite—or collagen, for that matter—as bones lay exposed to the elements.

At the time, Koch was finishing his Ph.D. thesis and was looking for a postdoctoral fellowship. He had been at Michigan for nearly seven years. By then, he was a full-fledged isotope paleoecologist, and he was eager to get on with his career.

Koch contacted Anna K. Behrensmeyer, a paleoecologist at the Smithsonian Institution. He knew that Behrensmeyer had been ex-

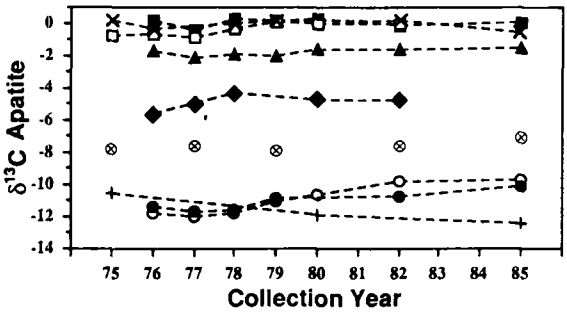


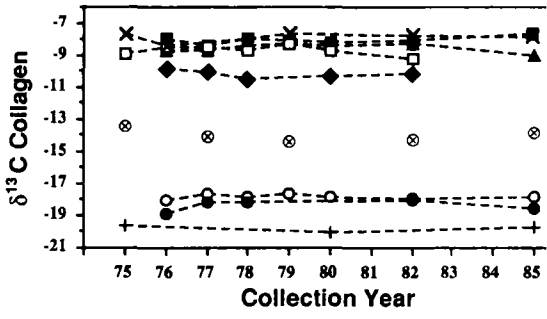
Koch and Behrensmeyer spent several weeks in Kenya's Amboseli Park in the summer of 1990. Top left: Mount Kilamanjaro looms majestically above the Park. Bottom left: Behrensmeyer with an elephant carcass. Top: Koch extracts an elephant tooth. (Photo credits: Bill Keyser)

amining the physical effects of weathering on bones as they lay exposed on the African savannah. Since 1975, she had been identifying and marking the carcasses of dead animals in Kenya's Amboseli National Park, gathering samples of bones from individual animals in succeeding years.

Behrensmeyer was intrigued by Koch's questions and suggested that he join her on her next collecting trip to Africa, in a few months hence. Koch applied for, and received, a postdoctoral fellowship at the Smithsonian; he also applied for a subsequent year of fellowship (later extended to two) at the Geophysical Laboratory, to which he was steered by a colleague of Behrensmeyer, Noreen Tuross, then working with Marilyn Fogel on the nursing study.

Koch, Behrensmeyer, and Behrensmeyer's husband arrived in Amboseli Park in September 1990. They stayed in a national park structure not far from a hotel used by tourists on safari. The hotel was full of modern amenities; their facility was not. It had a thatched roof teeming with bats and thick stone walls built to withstand foraging raids by elephants.





C3 feeder	Mixed feeder	C4 feeder
● Elephant	◆ Hippo.	▲ Zebra
○ Elephant	⊗ Impala	✕ Buffalo
+ *Rhino.*		■ *Ad. wildeb.*
		□ *Jv. wildeb.*

Carbon-isotope compositions of apatite (above left) and collagen (above right) in rib bones from seven Amboseli carcasses exposed to weathering vs. year collected. Ribs from the impala were unweathered and were used as a control. Koch's measurements showed that surface weathering caused negligible change to the carbon isotope values.

Koch and Behrensmeyer spent most of their time, from dawn to dusk, in the field cataloging, mapping, and collecting bones. Koch gathered some 400 bones from a great variety of animal carcasses, including zebras, hippopatami, rhinoceroses, elephants, wildebeests, and different species of gazelles. In addition, he and Behrensmeyer revisited and gathered bones from nearly all the carcasses Behrensmeyer had first sampled in 1975. Together, with the bones Behrensmeyer had collected in earlier years, each set of these weathered bones represented a progressively weathered sampling for an individual animal.

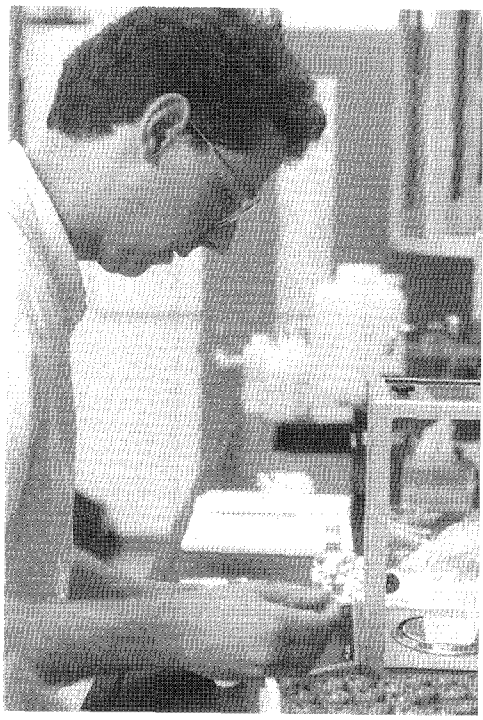
When Koch returned home, he brought the bones to the Geophysical Laboratory. He thought it possible there were directional changes with time in the weathered bones' apatite and collagen isotopes, reflecting isotope exchange with reservoirs of carbon, nitrogen, and oxygen in the ground and air. But he had no idea whether those changes, if they existed, would tend toward the heavy or light direction.

Koch spent the better part of a year analyzing the bones, isolating the collagen and apatite from each, converting both to gas, and then analyzing the gases in a mass spectrometer. The results surprised him. He found that virtually nothing was happening to the isotopes of either apatite or collagen (see graphs, above). If bones survive to be buried, he concluded, they will be buried with isotope values nearly intact; surface weathering causes no significant chemical decay. The alteration of apatite, then, occurs after burial.

On one hand, this was good news. It meant that he and other scientists working with isotope data wouldn't have to correct their data for alterations that may have occurred during surface exposure.

But it still left open the question of how useful bone apatite was in isotope analysis. Koch later participated in a study of animal skeletons that had been buried for hundreds to thousands of years. The researchers found that bone collagen and tooth enamel apatite gave constant, reasonable values, but that bone apatite was clearly altered. As a result, Koch says he now trusts apatite only in tooth enamel.

Since completing his bone decay study, Koch has branched off in three directions, one of them his interrupted dietary study of Pleistocene mastodons and mammoths. Though the study is far from complete, he has recently obtained some intriguing results, especially of nitrogen-isotope ratios. He found that mammoths in Michigan and New York had a heavier nitrogen isotope content than mastodons from the same time and place—a difference large enough to correspond to a step in the food chain. This was curious. Neither beast is a meat eater. To Koch this meant either that the mammoths were nutritionally stressed and were actually digesting their own tissues,* or that mastodons were eating some kind of nitrogen-fixing plants—which normally have lower nitrogen ratios than other plants—that mammoths were not. One strong possibility, he says, is that the mastodons were eating alder leaves. Alders were a common nitrogen-fixing deciduous tree in the Pleistocene.



Koch weighs a sample of mastodon tusk.

In another direction, Koch has also started to analyze other bones, as well as a variety of plants, that he gathered in Africa. These, he hopes, will provide a series of "snapshots" of the Amboseli ecosystem over the past twenty years. In those twenty years, Amboseli Park has changed a great deal, with the loss of nearly all woodlands. Whether this is a natural phenomenon or was caused at least partly by humans is unknown, but it is clear that such cycling—from grasslands to woodlands to grasslands—has been going on since at least the turn of the

*Koch relates that there is evidence in humans that a diet deficient in protein yields heavy nitrogen isotope ratios that are as high as three levels in the food chain. That evidence comes from an informal study that Noreen Tuross carried out on herself. Tuross ate plenty of C_3 plant foods but no C_4 plants and no animal protein (i.e., she had very little nitrogen) for over a year. At the end of that time, she noticed elevated nitrogen ratios in her fingernails. She immediately discontinued the study, warning her colleagues that high nitrogen ratios may mean something other than steps on the food chain.

Elephants are voracious eaters who may have been at least partially responsible for the change from woodlands to grasslands in the Amboseli ecosystem.

century. It may, he adds, even be caused by elephants, whose voracious appetite for leaves can easily strip a small forest in a few years.

Koch wants to see, via isotope ratios, what impact the changing vegetation at Amboseli has had on the diets of various animals. This, he hopes, will help him better understand his Pleistocene data. Africa today, he says, has a fauna that is a good analogy for the North American fauna at the end of the Pleistocene, when vegetation was also undergoing a radical shift.

Koch has also begun a new isotope project, studying a 55-million-year-old ecosystem in what is now Wyoming. During the summers of 1990 and 1991, he took time off from his bone decay study to spend a month in Wyoming gathering bone and tooth samples of ancient mammals, including the ancestors of horses.* He also gathered soil samples, as well as rocks containing traces of organic material that were once the plant material that the horses and other animals ate. He hopes to use isotopes from the fossil tooth apatite to learn something about the ancient climate. And he plans to use the organic molecules from plant material to assess the ancient atmospheric chemistry.

The questions involved in studying such an old environment are much different than those he is encountering in his studies of the more-recent Pleistocene. "There were no C_4 plants 55 million years ago," he says. "You have to look at subtle variations in carbon isotopes to learn about habitat."

Meanwhile, Koch is thinking about future career possibilities. He cannot remain a postdoctoral fellow for long; his grant will run out in 1993. He is having trouble, however, figuring out where he fits in the academic community. As an isotope paleoecologist, he sometimes

*Horses lived in North America until about 11,000 years ago, at which time they died out. The wild horses in North America today were introduced by European explorers.

feels as if he is neither a geologist nor a paleontologist. "I fall between the cracks," he says. All the same, his search has been fruitful. Just as this essay was going to press, he was offered and accepted an assistant professorship at Princeton University.

At Princeton, he hopes to continue exploring questions not only about the past, but about the present as well. Already he has become involved in the question of illegal elephant ivory trade in African parks. There is hope that isotopes may help determine the sources—and hence legality—of the ivory, though preliminary data, both Koch's and others, suggest the techniques are not yet ready to be applied. He would also like to become involved in conservation efforts, perhaps in assessing nutritional health of the parks' animal herds, again, by using isotope analyses.

Conclusion

The essays in this booklet have concentrated on one aspect of biogeochemistry—the application of amino acid and isotopic tools to the fields of anthropology and paleoecology. The topics are further limited to work ongoing at the Carnegie Institution of Washington, a small nonprofit science research and educational organization having only a dozen or so practicing biogeochemists, of whom only three hold staff member positions.

Yet the lessons to be learned go far beyond Carnegie's walls. Science is an endeavor that proceeds by individual efforts. Fifty years ago, few could have imagined that amino acids would provide a means of dating 100,000-year-old hominid sites. No one would have dreamed that stable isotopes would tell how long prehistoric peoples nursed their infants or what animals ate 10,000 years ago. Yet all of this can be traced to the contributions of a single individual, a single scientist poised to pursue a simple but critical question.

At the same time, science is nourished and fertilized by the spirit of collaboration. Had Philip Abelson's discovery not convinced others to work with him, to reproduce his results, to explore the ramifications of his finding, all might have been forgotten.

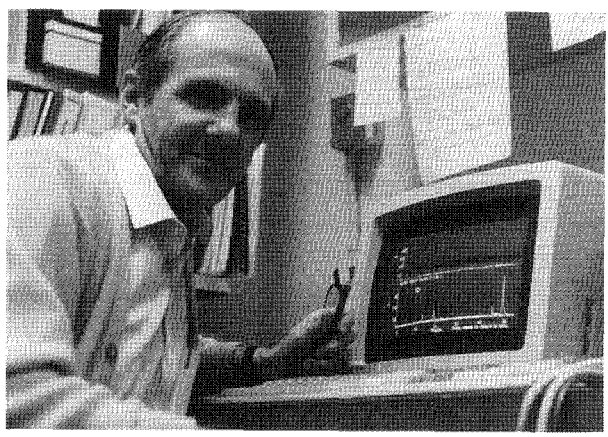
The future of biogeochemistry—at Carnegie and elsewhere—is bright. While many of its practitioners are interested in fundamental questions of organic decay, others are also interested in applying the techniques to problems that directly concern our lives and our environment. For example, some researchers are using stable isotopes to assess the purity of certain foods, such as honey and unsweetened orange juice, both derived from C_3 plants; samples with high ^{13}C values show the addition of cane or corn syrup. Likewise, brandy

and fortified wine are supposed to contain only products of the grape, a C_3 plant.

Other scientists are using isotopes to investigate metabolism and diet in humans. One unexpected result of Fogel and Tuross's study was the possibility of monitoring nutrition among patients requiring tube-feeding for long periods of time, such as stroke and coma victims, by measuring isotopes in fingernails.

Still other scientists, including Paul Koch, are working to develop isotope methods to help endangered animals. Others are doing the same using amino acids. Ed Hare, for example, has collaborated with scientists at Oregon's Fish and Wildlife Conservation Department in developing a quick method to date walrus ivory, using a fast-decaying amino acid.

In the future, the uses of isotope geochemistry will no doubt involve issues of global environmental change. For just as studies of present ecosystems promise to yield valuable information about past ecosystems—one of Paul Koch's goals—so, too, will studies of past ecosystems help scientists understand how our present environment reacts to change, both man-made and natural. The biogeochemists, at Carnegie and elsewhere, stand well poised to contribute to the challenges ahead.



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